Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 7434–7443
Bioorganic & Medicinal Chemistry

The molecular pruning of a phosphoramidate peptidomimetic inhibitor of prostate-specific membrane antigen

Lisa Y. Wu, Marc O. Anderson, Yoko Toriyabe, Jack Maung, Tammy Y. Campbell, Cheryl Tajon, Marat Kazak, Jamie Moser and Clifford E. Berkman*

Department of Chemistry & Biochemistry, San Francisco State University, 1600 Holloway Avenue, San Francisco, CA 94132, USA

Received 12 March 2007; revised 9 July 2007; accepted 10 July 2007
Available online 21 August 2007

Abstract—To identify the pharmacophore of a phosphoramidate peptidomimetic inhibitor of prostate-specific membrane antigen (PSMA), a small analog library was designed and screened for inhibitory potency against PSMA. The design of the lead inhibitor was based upon *N*-acyl derivatives of endogenous substrate folyl-γ-Glu and incorporates a phosphoramidate group to interact with the PSMA catalytic zinc atoms. The scope of the analog library was designed to test the importance of various functional groups to the inhibitory potency of the lead phosphoramidate. The IC_{50} for the lead phosphoramidate inhibitor was 35 nM while the IC_{50} values for the analog library presented a range from 0.86 nM to 4.1 μM. Computational docking, utilizing a recently solved X-ray crystal structure of the recombinant protein, along with enzyme inhibition data, was used to propose a pharmacophore model for the PSMA active site.

1. Introduction

A notable discovery in prostate cancer research has been the identification of an over-expressed membrane-bound cell surface protein on prostate cancer cells, namely, prostate-specific membrane antigen (PSMA). PSMA, also known as folate hydrolase I (FOLH1) and glutamate carboxypeptidase II (GCPII),[1–3] is a 750-amino acid type II membrane glycoprotein[4] and was discovered during the development of the LNCaP cell line; one which retains most of the known features of prostate cancer.[5]

Although PSMA is primarily expressed in normal human prostate epithelium, the importance of this enzyme lies with the fact that it is upregulated and strongly expressed in prostate cancer cells, including those of the metastatic disease state.[6] Recent studies have demonstrated PSMA expression in the endothelium of tumor-associated neovasculature of multiple nonprostatic solid malignancies,[7] including metastatic renal carcinoma.[8] As such, it is not surprising that PSMA has attracted a great deal of attention as a target for immunotherapy.[9–12] In addition to its immunological importance, PSMA is also reported to possess two predominant yet poorly understood enzymatic activities: the hydrolytic cleavage and liberation of glutamate from γ-glutamyl derivatives of folic acid[13,14] and the proteolysis of the neuropeptide *N*-acetylaspartylglutamate (NAAG).[2] However, recent studies suggest that PSMA plays a regulatory role in angiogenesis.[15]

Until recently,[16,17] no crystal structure of PSMA was available and thus the development of specific inhibitors relied upon rational strategies to identify and exploit binding sites in the structural landscape proximal to the active site. For example, we recently identified the presence of a hydrophobic binding site remote from the catalytic center of PSMA using a series of phenylalkylphosphonamidate derivatives of glutamic acid as hydrophobic probes.[18] In addition, we found that a phenylazobenzoyl derivative of γ-diglutamate was substantially equivalent to NAAG as a substrate for PSMA.[18] These results led us to hypothesize that a tetrahedral intermediate analog to the hydrolysis of a *N*-acyl γ-diglutamate should exhibit reasonable inhibitory potency against PMSA. Herein we explore the use of substituted phosphoramidates as transition-state analog inhibitors of PSMA.

Keywords: Prostate-specific membrane antigen (PSMA); Glutamate carboxypeptidase II; Phosphoramidate; Peptidomimetic.
* Corresponding author. Tel.: +1 415 338 1288; fax: +1 415 338 2384; e-mail: cberkman@sfsu.edu

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.07.028

2. Results and discussion

The initial design of a phosphoramidate-based PSMA inhibitor is exemplified by lead compound **1a** (Fig. 1). This compound incorporates features of endogenous PSMA substrate folyl-γ-Glu, namely the L-Glu residue at P1′, a carboxyl substituent at the P1 position, and a benzamide group on the P1-amino terminus. The P1 position contains a serine residue, which is conveniently conjugated to a phosphoramidate functionality, serving as a zinc-binding group for the PSMA active site zinc atoms. Importantly, the phosphoramidate group was positioned to act as a transition-state mimic of the scissile amide bond in folyl-γ-Glu.

In addition to phosphoramidate **1a**, which most closely resembles the folyl-γ-Glu substrate, a series of analogs was also explored in order to examine structural and functional features that may be necessary for PSMA inhibitory activity. To this end, a series of novel compounds (**1b**–**1k**), as well as the known inhibitor 2-PMPA (**1l**)[19], were prepared and examined for inhibitory potency against purified PSMA (Fig. 2).

A general method for the synthesis of phosphoramidates **1a**–**1h** is outlined in Scheme 1. *N*,*N*-Bis(diisopropylamino)-chlorophosphine **2** was treated with benzyl alcohol to generate *O*-benzyl substituted phosphine **3**. This species was treated with various primary alcohols **4a**–**4h** and diisopropylammonium tetrazolide to generate the dialkoxy substituted phosphines **5a**–**5h**. Next, hydrolysis was mediated by 5-ethylthio-1*H*-tetrazole to form dialkoxy substituted phosphites **6a**–**6h**. Oxidative coupling with bis-benzyl protected glutamic acid, through the transient phosphoryl-chloride species, leads to protected phosphoramidate species **7a**–**7h**. Each of these protected phosphoramidates was purified by silica gel flash chromatography and appeared to be homogeneous by TLC analysis as well as ^{1}H and ^{13}C NMR. Lastly, deprotection was carried out by hydrogenolysis in potassium carbonate buffered solution to form the potassium salts of the final products **1a**–**1h**. We have found phosphoramidates to be quite unstable to acid and that the compounds readily decompose in their protonated forms. As such, the final products were generally isolated as cationic salt forms. To confirm that the compounds would be stable under the conditions of the PSMA inhibition experiments, we monitored the stability of a representative analog (**1a**) at pH 6.8, 7.4, and 8.6 by ^{31}P NMR every 15 min for 6 h. No change was observed for **1a** at pH 7.4 and 8.6, while at pH 6.8 it underwent hydrolysis to **1i** with a half-life of 408 min. Based upon these results, we concluded that the inhibitors would be sufficiently stable under the conditions

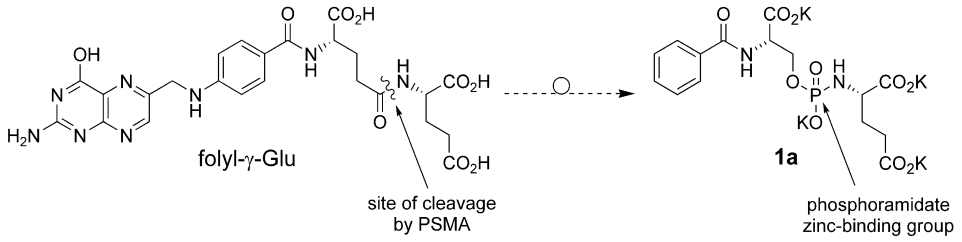


Figure 1. Design of lead phosphoramidate peptidomimetic **1a**.

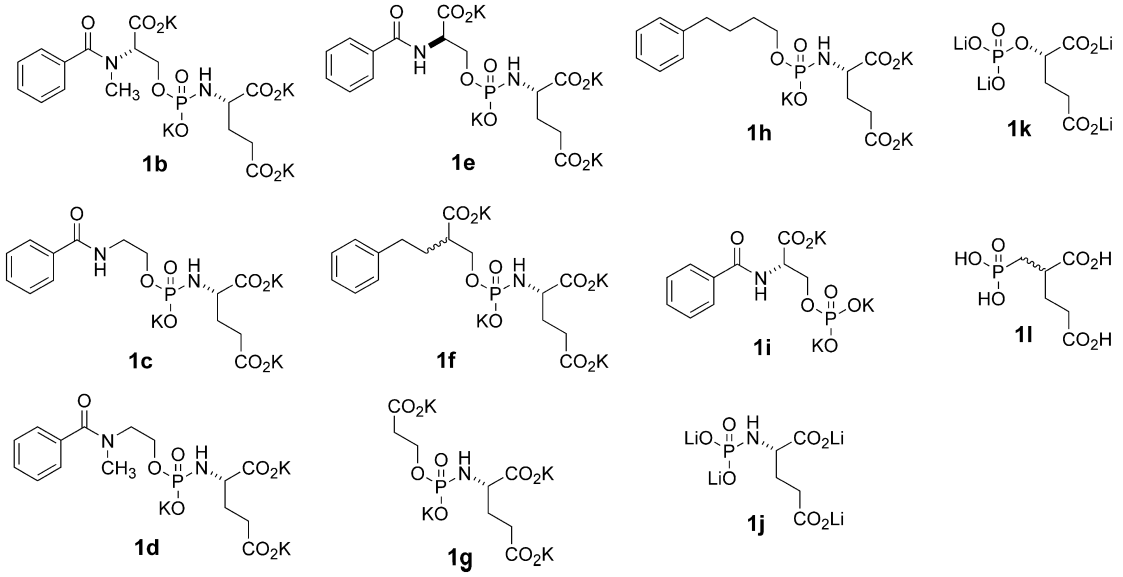
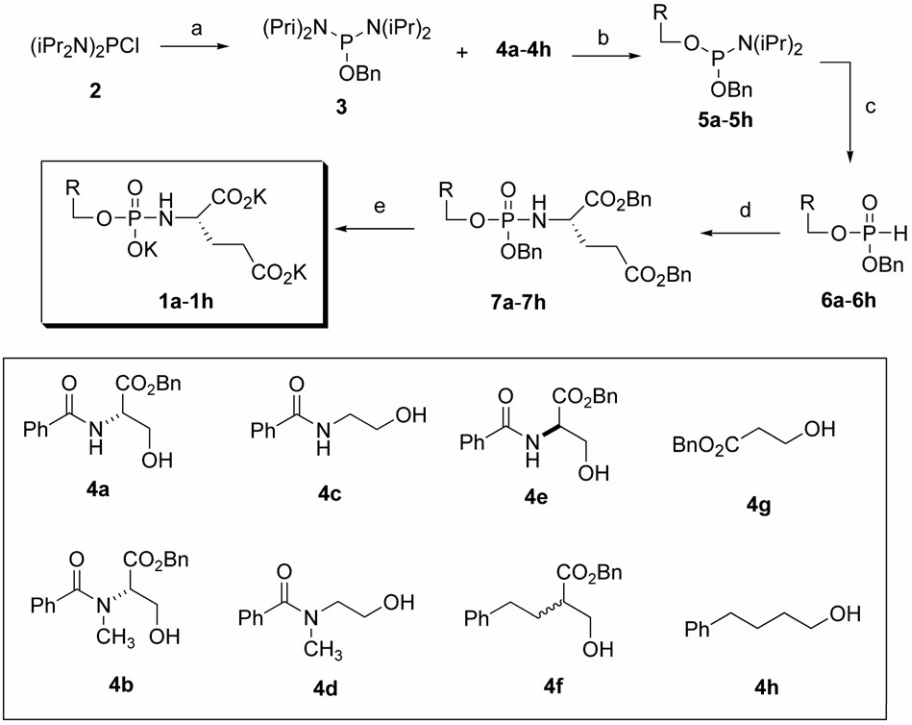


Figure 2. Analogs of peptidomimetic **1a** based on phosphoramidate (**1b**–**1h**, **1j**), phosphate (**1i**, **1k**), and phosphonate (**1l**) scaffolds.

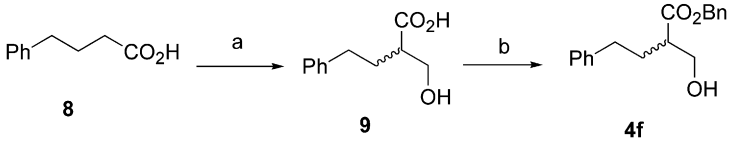


Scheme 1. General synthesis of phosphoramidate inhibitors that incorporate primary alcohols at the P1 position. Reagents and conditions: (a) benzyl alcohol (1.0 equiv), Et_3N (3.0 equiv), CH_2Cl_2, 0 °C, 10 min 1 h at rt; (b) diisopropylammonium tetrazolide (1.05 equiv), primary alcohols **4a**–**4i**, CH_2Cl_2, 3 h at rt; (c) 5-ethyl-thiotetrazole (1.10 equiv), CH_3CN, H_2O; (d) CH_3CN, Et_3N (2.6 equiv), *p*-TsOH H-Glu(OBn)-OBn (1.3 equiv); then CCl_4 (10 equiv), 2 h at rt; (e) H_2, *cat.* Pd (10% on C), K_2CO_3, THF-H_2O, 3 h, rt.

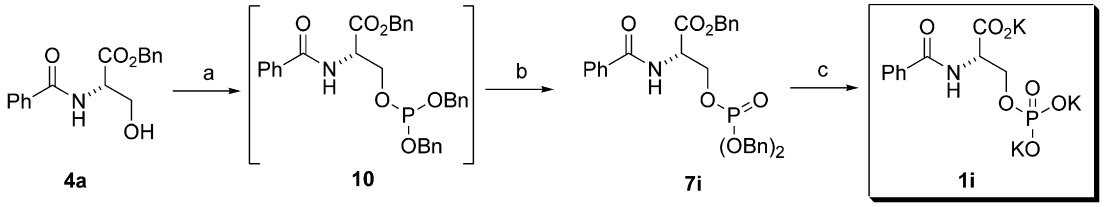
of the enzyme inhibition assay (pH 7.4). These results are in agreement with our previous findings for simple alkyl phosphoramidates.[20]

In general, the primary alcohol building blocks **4a**–**4h** were either commercially available or prepared by simple protecting group attachment (typically benzyl esters) to available species. One exception is the building block **4g**, which was generated from the base-mediated opening of β-propiolactone with benzyl alcohol as was previously reported.[21] Another exception was species **4f**, which was prepared as shown in Scheme 2. Thus, 4-phenyl-butyric acid (**8**) was converted to the corresponding dianion by treatment with LDA (2 equiv) and then trapped with formaldehyde[22] to generate β-hydroxy acid **9**. Subsequent protection yielded the β-hydroxyl benzyl ester species **4f**.

The preparation of compound **1i** is outlined in Scheme 3. Protected serine derivative **4a** was coupled with a dibenzyl protected phosphoramidite species to generate intermediate **10**, which was subsequently oxidized to



Scheme 2. Synthesis of protected β-hydroxy ester **4f**. Reagents and conditions: (a) LDA (2.1 equiv), THF, −78 °C then formaldehyde from thermolysis of paraformaldehyde (6.0 equiv); (b) benzyl bromide (1.1 equiv), Cs_2CO_3 (0.55 equiv), DMF.



Scheme 3. Synthesis of compound **1i**, lacking a P1′ glutamate residue. Reagents and conditions: (a) dibenzyl diisopropylamino phosphoramidite, 5-ethylthio-1*H*-tetrazole, CH_3CN, 9 h, rt; (b) *tert*-butylhydroperoxide, 45 min, rt; (c) H_2, *cat.* Pd (10% on C), K_2CO_3, THF-H_2O, 3 h, rt.

Table 1. IC_{50} values for analogs of phosphoramidate **1**[a]

Inhibitor	IC_{50} (nM)
1a	35 (6)
1b	55 (6)
1c	1800 (200)
1d	280 (30)
1e	700 (71)
1f	790 (90)
1g	4100 (310)
1h	180 (5)
1i	ND[b]
1j	0.86 (0.7)
1k	15 (1)
1l	2.3 (0.3)

[a] Error in parentheses.
[b] No detectable inhibition at inhibitor concentrations 10 μM or less.

protected phosphate **7i**. This was deprotected under hydrogenolysis conditions to generate the desired phosphate conjugated serine species **1i**. Phosphoramidate **1j**, phosphate **1k**, and racemic phosphonate **1l** (aka. 2-PMPA) were prepared as described previously.[19,20,23]

Once obtained, compounds **1a**–**1l** were assayed for inhibition against purified PSMA (Table 1) as described previously.[24,18,25] Screening of this library indicated that two general structural frameworks displayed superior inhibitory potency: intact phosphoramidate peptidomimetics such as **1a** and **1b** as well as simple P1′ analogs **1j**–**1k** and **1l** (2-PMPA).[19] Despite the lack of additional affinity elements, we hypothesized that the dibasic phosphoryl or phosphonyl motif of the latter three compounds is responsible for their enhanced affinity for PSMA through strong interactions with PSMA's active-site zinc atoms. The des-glutamate analog **1i** was void of activity confirming that a C-terminal glutamate residue confers considerable binding specificity for PSMA. Compared to the carboxylate analog **1g**, the phenylalkyl phosphoramidate **1h** retained significant inhibitory potency against PSMA suggesting that the binding landscape or the N-terminal side of the catalytic center is more favorable toward hydrophobic motifs. With respect to the structure of the lead phosphoramidate peptidomimetic **1a**, the stereoisomeric analog **1e** confirmed the stereochemical importance of the P1 residue. An interesting effect was observed in cases where secondary benzamides (**1a** and **1c**) were compared with their tertiary congeners (**1b** and **1d**). In the case of the serine derivatives (**1a**–**1b**), the presence of an extra amide methyl group induced a marginal decrease in activity, while for the 2-aminoethanol derivatives (**1c**–**1d**) the same modification resulted in a more potent inhibitor. As tertiary amides typically exist as mixtures of rotamers, it is possible that the *cis* and *trans* forms of **1b** and **1d** have differential activity against PSMA. Furthermore, this implies that the IC_{50} values for these inhibitors are averaged over the populations of *cis* and *trans* rotameric forms present in the assay conditions. Given the greatly improved potency of **1d** over **1c**, and its increased likelihood to partition into the *cis* rotameric form, we speculate that this rotamer may explain the improved potency of **1d** relative to **1c**, while the effect may be less pronounced or opposite in the case of **1b** relative to **1a**.

In order to shed further insight into the complementary interactions of these compounds with the active site landscape, computational docking of three representative inhibitors was carried out employing a recently determined X-ray crystal structure of PMSA.[16] The docking results of *N*-benzamido serine derived phosphoramidate inhibitor (**1a**) are depicted in Fig. 3a. The key binding features of this inhibitor are the glutamate α-carboxyl which interacts with Arg^{210}, Tyr^{700}, and Tyr^{552}, while the γ-carboxyl interacts with Asn^{257} and Lys^{699}. The phosphoramidate oxygens form a chelated complex with the active site zinc atoms. The serine α-carboxyl interacts with Arg^{536} while the attached benzamide group is aligned to make potential π-stacking and hydrophobic interactions with active site tyrosine residues. The docked conformation of ethanolamine-linked phosphoramidate inhibitor (**1d**) is shown in Fig. 3b. Overall, this inhibitor maintains a conformation consistent with **1a**, with the same interactions maintained by the glutamate α- and γ-carboxyls, as well as the interactions of the benzamide functionality. An additional interaction between the benzamide carbonyl is made with Arg^{536}. A notable difference between inhibitors **1a** and **1d** is the lack of the serine α carboxyl interaction in **1d**, which may explain its significantly decreased potency when compared to compound **1a**. Lastly, the docking results of potent phosphorus-conjugate glutamate inhibitor **1j** are shown in Fig. 3c. The glutamate γ-carboxyl maintains similar interactions as were observed for compounds **1a** and **1d**. As expected, the α-carboxyl is again poised for interaction with Arg^{210}, yet the structure of this compound also allows this functional group to participate in chelated interactions with the active site zinc atoms. This, combined with an extra available phosphoramidate oxygen interaction with zinc, could explain the significantly improved potency of **1j** in comparison with inhibitors **1a** and **1d**.

Based upon experimental and docking results, we propose a pharmacophore model that defines several inhibitor features important for activity. Most importantly, an L-Glu residue appears important at P1′ to maintain an interaction between the inhibitor γ-carboxylate and Asn^{257} and Lys^{699}, as well as an interaction between the α-carboxylate group with Arg^{210}. Secondly, a zinc-binding group (ZBG) is important, which in this series of molecules is a phosphoramidate, phosphate, or phosphonate functionality. A P1 group is optional for activity but, if present, should contain hydrophobic functionality, preferably an aromatic ring tethered within four atoms of the ZBG to interact in π-stacking or hydrophobic interactions with nearby aromatic residues Tyr^{234}, Tyr^{549}, Tyr^{552}, and Tyr^{700}. Lastly, a P1 carboxylate provides additional interactions with Arg^{536}, while a hydrophilic carboxylate group alone in P1 is unsatisfactory and may compete for the Arg^{210}/Lys^{699} interactions in S1′, thus reversing the mode of binding.

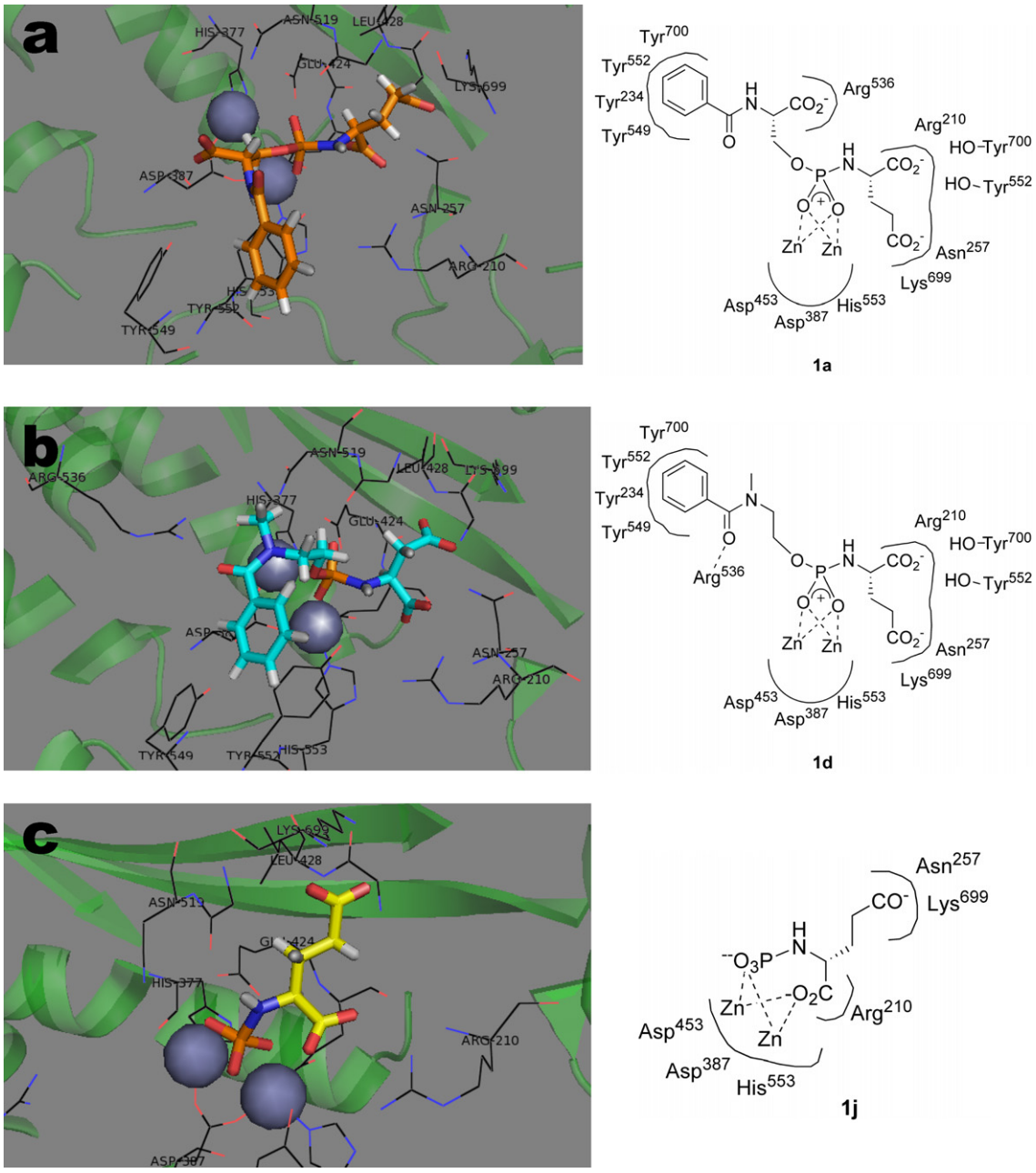


Figure 3. Computational docking of phosphoramidate inhibitors into the active site of PSMA: panel a, inhibitor **1a** (orange); panel b, inhibitor **1d** (cyan); panel c, inhibitor **1j** (yellow). In each panel, the figure on the left is a graphical representation showing the docked configuration of the inhibitor in the active site (PDB = 2C6C); the figure on the right is a cartoon representation showing key interactions of substrates with the nearby residues.

3. Conclusion

A lead phosphoramidate peptidomimetic (**1a**) was designed to mimic the tetrahedral intermediate of the hydrolysis of a *N*-acyl diglutamate substrate by PSMA, and was found to be a potent inhibitor of this enzyme. Based upon the structural framework of this inhibitor, a small library of compounds (**1b**–**1k**) was prepared to explore the pharmacophore requirements of the PSMA active site. Inhibitory potency was determined for **1a**–**1k**, along with the known inhibitor 2-PMPA (**1l**). Basic pharmacophore requirements were determined based on the inhibitory data for these compounds, and a tentative mode of binding for inhibitors **1a**, **1d**, and **1j** was suggested based on computational docking studies utilizing a recent crystal structure of PSMA. The development of further optimized inhibitors based upon these conclusions will be reported in due course.

4. Experimental

4.1. Synthesis

All solvents used in reactions and diisopropylethylamine (DIPEA) were both anhydrous and obtained as such

from commercial sources. All other reagents were used as supplied unless otherwise stated. Liquid flash chromatography (silica or C18) was carried out using a Biotage 12i/40i system. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on either a Bruker 300 or 500 MHz instrument. ^{1}H NMR chemical shifts are relative to TMS ($\delta = 0.00$ ppm), $CDCl_3$ ($\delta = 7.26$ ppm), CD_3OD ($\delta = 4.87$ and 3.31 ppm), or D_2O ($\delta = 4.87$). ^{13}C NMR chemical shifts are relative to CD_3OD ($\delta = 49.15$ ppm) or $CDCl_3$ ($\delta = 77.23$ ppm). ^{31}P NMR chemical shifts in $CDCl_3$, CD_3OD, or D_2O were externally referenced to 85% H_3PO_4 ($\delta = 0.00$ ppm) in $CDCl_3$, CD_3OD, and D_2O, respectively. High resolution mass spectra (FAB) were performed by the University of Notre Dame Mass Spectrometry Facility, Notre Dame, IN 46556-5670.

4.2. General procedure A: Synthesis of benzyl-protected precursors to phosphoramidate inhibitors. *N*2-[(2-Benzoylamino-2-benzyloxycarbonyl-ethoxy)-benzyloxy-phosphorylamino]-pentanedioic acid dibenzyl ester (7a–7h)

Triethylamine (590 μL, 4.18 mmol) and benzyl alcohol (1 equiv) were sequentially added dropwise to a stirred solution of bis(diisopropylamino) chlorophosphine (1 equiv) in anhydrous hexane (35 mL). The reaction mixture was stirred for 3 h at rt, after which the solids were filtered and solvent was removed in vacuo. The residue was immediately dissolved in anhydrous CH_2Cl_2 (15 mL) and transferred via syringe to a stirred solution of an alcohol (**4a**–**4i**) and *N*,*N*-diisopropylammonium tetrazolide (0.6 equiv) in CH_2Cl_2 (20 mL) at 0 °C under argon. The reaction mixture was stirred 30 m at rt, after which the solids were filtered and solvent was removed in vacuo. The crude reaction mixture was dissolved in CH_3CN (30 mL) and cooled to 0 °C. A solution of 1-*H*-tetrazole (1 equiv) in a mixture of distilled H_2O (1 mL) and CH_3CN (1 mL) was then added and the reaction mixture stirred for 15 min at 0 °C and then 1 h at rt. Solvent was removed in vacuo. The residue was washed twice with 10% HCl (50 mL) and the crude mixture was extracted with EtOAc (50 mL). The organic layer was sequentially washed with 10% $NaHCO_3$ (50 mL), distilled H_2O (50 mL), and brine (50 mL). After drying the organic layer with $MgSO_4$, the solvent was removed in vacuo to yield the phosphite (**6a**–**6i**), typically as an oil, which was used immediately in the next step without characterization or purification.

A solution of glutamic acid dibenzyl ester (0.475 g, 1.45 mmol) in CH_3CN (6 mL) was added dropwise to a stirred solution of crude phosphite (0.9 equiv) in CH_3CN (3 mL) and CCl_4 (6 mL) at 0 °C under argon. The reaction mixture was stirred for 2 h and solvent was reduced to half its volume in vacuo. The residue was dissolved in CH_2Cl_2 (40 mL) and sequentially washed with 10% HCl (2×40 mL), 10% $NaHCO_3$ (40 mL), and brine (40 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give a yellow oil. The product was isolated by flash chromatography (1:1 hexane/ethyl acetate). Rf ≈ 0.3. The isolated product could be further purified by recrystallization from benzene/cyclohexane (1:5 v/v) to generate benzyl protected phosphoramidates (**7a**–**7h**).

4.3. General procedure B: Deprotection to generate phosphoramidate inhibitors as potassium salts (1a–1i)

To a solution of a benzyl ester protected phosphoramidate (**7a**–**7h**) or phosphate (**7i**) (0.254 mmol) in THF (2 mL) were added 10% Pd/C (20 mg), K_2CO_3 (2 equiv), and distilled H_2O (2 mL). The mixture was stirred vigorously, purged with argon, and hydrogenated overnight at rt. The solvent was removed in vacuo and the residue was dissolved in methanol, filtered through Celite and then through a 0.2 μm PTFE micropore filtration disk (Whatman). Solvent was removed in vacuo to yield a viscous oil (**1a**–**1i**). In some compounds, triteration with ethyl acetate could be used to generate products in solid form.

4.4. *N*-Benzoyl-Ser-[PO_2Bn-NH-Glu(OBn)-OBn]-OBn (7a) (using general procedure A)

Yield 28%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.84–1.96 (m, 2H), 2.03–2.14 (m, 1H), 2.27–2.46 (m, 2H), 3.45 (t, 1H, $J = 20.11$ Hz), 3.88–3.98 (m, 1H), 4.34–4.54 (m, 2H), 4.83–5.21 (m, 8H), 7.26–7.52 (m, 23H), 7.94 (d, 2H, $J = 7.23$ Hz), 8.28 (d, 1H, $J = 7.4$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.63, 30.38, 54.40, 54.50, 67.15, 67.22, 68.10, 68.18, 69.29, 69.38, 128.06, 128.18, 128.36, 128.94, 129.04, 129.16, 129.23, 132.38, 134.04, 135.63, 135.93, 136.27, 136.33, 136.43, 167.33, 169.10, 172.41, 172.49. ^{31}P NMR (121 MHz, $CDCl_3$): δ 6.35 FAB-HRMS $(M+H)^+$ Calcd 779.2734. Found: 779.2710 for $C_{43}H_{44}N_2O_{10}P$.

4.5. *N*-Benzoyl-Ser-[PO_2K-NH-Glu(OK)-OK]-OK (1a) (derived from 7a using general procedure B)

Yield 89%. ^{1}H NMR (300 MHz, D_2O): δ 1.76–1.91 (m, 2H), 2.15–2.26 (m, 2H), 3.49–3.55 (m, 1H), 4.19 (t, 2H, $J = 5$ Hz), 4.54 (t, 1H, $J = 8.9$ Hz), 7.53–7.67 (m, 3H), 7.89 (d, 2H, $J = 7.6$ Hz). ^{13}C NMR (75 MHz, D_2O): δ 32.72, 34.71, 57.56, 57.67, 65.68, 128.30, 129.64, 133.08, 134.23, 171.11, 176.97, 182.44, 183.79. ^{31}P NMR (121 MHz, D_2O): δ 8.10 FAB-HRMS $(M-K)^-$ Calcd 530.9376. Found: 530.9372 for $C_{15}H_{15}K_3N_2O_{10}P$.

4.6. *N*-Benzoyl-*N*-methylserine-[PO_2Bn-NH-Glu(OBn)-OBn]-OBn (7b) (using general procedure A)

Yield 42%. ^{1}H NMR (300 MHz, acetone-d_6, two rotamers 1:1): δ 1.87–1.97 (m, 1H), 2.10–2.17 (m, 1H), 2.42–2.57 (m, 2H), 2.42–2.57 (m, 2H), 2.93 (s, 2H), 2.95 (s, 1H), 3.94–4.02 (m, 1H), 4.54–4.57 (m, 1H, minor), 4.52–4.63 (m, 1H), 4.81 (t, 1H, $J = 12$ Hz), 4.95–5.23 (m, 8H), 7.26–7.36 (m, 25H). ^{13}C NMR (75 MHz, acetone-d_6 and CD_2Cl_2 two rotamers): δ 29.67 and 29.73, 30.17 and 30.40 and 30.45, 36.77 and 37.36, 54.70, 59.29 and 59.75 and 60.69, 63.87 and 64.22, 66.74, 67.63, 68.62 and 68.70, 68.81, 128.00, 128.48 and 128.55, 128.96 and 129.04, 129.11 and 129.34, 130.31 and 130.47, 136.10, 137.01 and 137.22, 168.94, 172.97, 173.15. ^{31}P NMR (121 MHz, acetone-d_6) δ 10.00 and 9.88 FAB-HRMS $(M+H)^+$ Calcd 793.2898. Found: 793.2872 for $C_{44}H_{46}N_2O_{10}P$.

4.7. *N*-Benzoyl-*N*-methylserine-[PO_2K-NH-Glu(OK)-OK]-OK (1b) (derived from 7b using general procedure B)

Yield 85%. ^{1}H NMR (300 MHz, D_2O; two rotamers, major/minor = 1.7:1): δ 1.80–1.89 (m, 2H), 2.14–2.23 (m, 2H), 3.00 (s, 1H), 3.05 (s, 2H), 3.46–3.49 (m, 1H), 3.97–4.11 (m, 2H), 4.14–4.33 (m, 1H), 4.47–4.52 (m, 2H), 5.00–5.05 (m, 1H), 7.44–7.54 (m, 5H). ^{13}C NMR (75 MHz, D_2O; two rotamers): δ 30.00 and 30.06, 32.31 and 33.98, 34.07 and 35.66, 35.72, 56.79 and 56.89 and 57.04, 61.03 and 61.13, 61.86, 62.24, 65.03, 65.10 and 65.19, 126.93 and 127.02, 128.94 and 129.09, 130.32 and 130.38, 135.27, 135.66, 161.32, 174.64, 174.73, 174.96, 175.59, 181.42 and 181.48, 181.66 and 181.71, 183.21. ^{31}P NMR (121 MHz, D_2O; two rotamers): δ 7.36 and 7.58 FAB-HRMS $(M+K)^+$ Calcd 622.8806. Found: 622.8837 for $C_{16}H_{17}K_5N_2O_{10}P$.

4.8. 2-[(2-Benzoylamino-ethoxy)-benzyloxy-phosphorylamino]-pentanedioic acid dibenzyl ester (7c) (using general procedure A)

Yield: 25%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.93–2.02 (m, 2H), 2.36–2.44 (m, 2H), 3.63–3.70 (m, 3H), 4.10–4.13 (m, 3H), 4.94–5.09 (m, 6H), 7.25–7.88 (m, 20H), 7.85–7.88 (d, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.86, 30.38, 30.45, 41.21, 54.36, 54.53, 66.14, 66.76, 68.00, 69.13, 69.19, 77.70, 127.82, 129.21, 129.96, 134.72, 136.23, 136.30, 136.45, 136.65, 167.96, 168.02, 173.11, 173.12, 173.17. ^{31}P NMR (121 MHz, $CDCl_3$): δ 9.55. FAB-HRMS $(M+H)^+$ Calcd 645.2366. Found: 645.2337 for $C_{35}H_{38}N_2O_8P$.

4.9. 2-[(2-Benzoylamino-ethoxy)-hydroxy-phosphorylamino]-pentanedioic acid tripotassium salt (1c) (derived from 7c using general procedure B)

Yield 75%. ^{1}H NMR (300 MHz, D_20): δ 1.77 (q, 2H, J = 7.42 Hz), 2.09 (t, 2H, J = 8.15 Hz), 3.38 (dt, 2H, J = 8.79 Hz), 3.49 (t, 1H, J = 5.12 Hz), 3.83 (dt, 2H, J = 5.88 Hz), 7.41–7.70 (m, 5H). ^{13}C NMR (75 MHz, D_20): δ 31.55, 31.63, 33.46, 40.65, 40.75, 56.60, 62.74, 62.81, 127.05, 128.62, 131.96, 133.45, 170.93, 181.40, 181.46, 182.51. ^{31}P NMR (121 MHz, D_20): δ 8.54. FAB-HRMS $(M-K)^-$ Calcd 448.9918. Found: 448.9916 for $C_{14}H_{16}K_2N_2O_8P$.

4.10. 2-{[2-(Benzoyl-methyl-amino)-ethoxy]-benzyloxy-phosphorylamino}-pentanedioic acid dibenzyl ester (7d) (using general procedure A)

Yield 31%. ^{1}H NMR (300 MHz, $CDCl_3$). Note: while isolated as a homogeneous species, ^{1}H NMR characterization of this compound was complicated due to extensive broadening caused by *N*-rotomerism in the presence of *P*-diastereomers. ^{13}C NMR (75 MHz, $CDCl_3$ two rotamers and two diastereomers): δ 29.72, 29.79, 30.39, 38.97, 48.26, 48.32, 64.40, 66.77, 67.66, 68.53, 68.58, 68.64, 127.42, 128.17, 128.22, 128.63, 128.78, 128.87, 129.00, 129.05, 129.86, 136.05, 136.60, 137.06, 172.02, 172.79, 173.13, 173.19. ^{31}P NMR (121 MHz, $CDCl_3$): δ 8.30 and 8.38 FAB-HRMS $(M+H)^+$ Calcd 659.2522. Found: 659.2549 for $C_{36}H_{40}N_2O_8P$. Anal. Calcd for $C_{36}H_{39}N_2O_8P$: C, 65.64; H, 5.97; N, 4.25. Found: C, 65.58; H, 5.67; N, 4.31.

4.11. 2-{[2-(Benzoyl-methyl-amino)-ethoxy]-hydroxy-phosphorylamino}-pentanedioic acid tripotassium salt (1d) (derived from 7d using general procedure B)

Yield 82%. ^{1}H NMR (300 MHz, D_2O, two rotamers, major/minor = 1.1:1): δ 1.80–1.92 (m, 2H), 2.13–2.24 (m, 2H), 3.06 (s, 1.4H), 3.14 (s, 1.6H), 3.44–3.57 (m, 2H), 3.76–3.84 (m, 2H), 4.03 (q, 2H, J = 5.7 Hz), 7.44–7.53 (m, 5H). ^{13}C NMR (75 MHz, D_2O two rotamers): δ 31.82 and 31.85, 33.28 and 33.55 and 33.66, 38.82, 48.18 and 48.29, 51.50 and 51.61, 56.41 and 56.56, 61.33 and 61.40 and 61.70 and 61.77, 126.43 and 126.45, 128.55 and 128.64, 129.79 and 129.97, 134.96 and 135.11, 173.84 and 174.72, 181.18 and 181.28, 182.81 and 182.86. ^{31}P NMR (121 MHz, D_2O): δ 7.43 and 7.72 (externally referenced with *O*-phosphoric acid). FAB-HRMS $(M-H)^-$ Calcd 500.9634. Found: 500.9631 for $C_{15}H_{17}K_3N_2O_8$ P.

4.12. *N*-Benzoyl-D-Ser-[PO_2Bn-NH-Glu(OBn)-OBn]-OBn (7e) (using general procedure A)

Yield 57%. ^{1}H NMR (300 MHz, $CDCl_3$, two rotomers): δ 1.80–2.05 (m, 2H), 2.24–2.41 (m, 2H), 4.22–4.38 (m, 1H), 4.80–4.95 (m, 2H), 5.01–5.14 (m, 8H), 5.07–5.10 (m, 1H), 7.20–7.40 (m, 24H), 7.74 (d, J = 7.02 Hz, 2H), 7.82 (d, J = 6.28 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.65, 30.48, 53.65, 55.27, 63.27, 66.26, 66.78, 67.46, 68.53, 68.59, 127.17, 128.21, 128.35, 128.43, 128.52, 128.56, 128.68, 131.76, 131.82, 133.56, 135.13, 135.48, 135.62, 135.82, 167.58, 170.50, 172.32, 172.83. ^{31}P NMR (121 MHz, $CDCl_3$, two rotomers and two diastereomers): δ 5.79 and 6.30 (externally referenced with *O*-phosphoric acid). HRMS (FAB+) Calcd [M+1] = 779.2733. Found: [M+1] = 779.2728 for $C_{43}H_{43}N_2O_{10}P$.

4.13. *N*-Benzoyl-D-Ser-[PO_2K-NH-Glu(OK)-OK]-OK (1e) (derived from 7e using general procedure B)

Yield 55%. ^{1}H NMR (300 MHz, D_2O): δ 1.27 (t, J = 7.31 Hz, 6H), 1.95–2.20 (m, 2H), 2.42 (t, J = 6.58, 2H), 3.07 (q, J = 7.21 Hz, 4H), 3.57–3.65 (m, 1H), 3.99–4.11 (m, 2H), 4.42–4.46 (m, 1H), 7.43–7.65 (m, 7H), 7.83 (d, J = 7.16 Hz, 2H). ^{13}C NMR (75 MHz, D_2O): δ 10.37, 24.01, 28.99, 42.08, 52.93, 55.35, 55.86, 60.77, 63.75, 127.20, 128.63, 132.39, 132.66, 170.94, 172.19, 174.05, 174.29. ^{31}P NMR (121 MHz, D_2O): δ 5.24 (externally referenced with *O*-phosphoric acid). HRMS (FAB+) Calcd [M+1] = 570.9091, found [M+1] = 570.9087 for $C_{15}H_{15}O_{10}N_2PK_4$.

4.14. 2-Hydroxymethyl-4-phenyl-butyric acid benzyl ester (4f)

Anhydrous THF (20 mL) was cooled to −78 °C and treated with LDA (1 M/THF) (12.8 mL, 12.8 mmol). The solution was stirred at −78 °C and then a solution of 4-phenyl-butyric acid (1.0 g, 6.09 mmol, 0.48 equiv) in THF (3 mL) was added dropwise over 1 min. The

solution was stirred for 5 min, and was then treated with HMPA (2.1 mL), stirred for 5 more minutes at −78 °C, and then stirred in an ice bath for 10 min. The reaction mixture was then treated with a stream of anhydrous formaldehyde over a period of ~30 min, led in from another reaction vessel containing paraformaldehyde (1.09 g, 36.5 mmol, 6.0 equiv). The reaction mixture was then treated with NH_4Cl (satd aq), acidified to pH 4, and extracted with EtOAc (3 × 15 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to yield 2-hydroxymethyl-4-phenyl-butyric acid (**9**) as a slightly greenish-yellow oil (0.904 g, 76%) which was used in the next step without purification or characterization.

To a solution of crude 2-hydroxymethyl-4-phenyl-butyric acid (**9**) (0.904 g, 4.65 mmol) in DMF (15 mL) were added Cs_2CO_3 (0.834 g, 2.55 mmol, 0.55 equiv) and allyl bromide (0.61 mL, 5.12 mmol, 1.1 equiv). The solution was stirred for 1 h and then treated with H_2O (30 mL), and the crude product was extracted with Et_2O (3 × 15 mL). The organic material was purified with silica gel flash chromatography to yield the product 2-hydroxymethyl-4-phenyl-butyric acid benzyl ester (**4f**) as a colorless oil (0.469 g, 35%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.85–2.06 (mult, 2H), 2.26 (br s, 1H), 2.61–2.69 (mult, 3H), 3.78–3.82 (mult, 2H), 5.18 (d, 2H, J = 3 Hz), 7.13 (d, 1H, J = 6 Hz), 7.25–7.28 (mult, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 30.1, 33.2, 47.0, 63.0, 63.4, 126.0, 128.3, 128.5, 135.7, 141.1, 155.3, 174.8.

4.15. 2-[Benzyloxy-(2-benzyloxycarbonyl-4-phenyl-butoxy)-phosphorylamino]-pentanedioic acid dibenzyl ester (7f) (using general procedure A)

^{1}H NMR (300 MHz, MeOH-d_4): δ 1.65–1.93 (m, 3H), 2.01–2.13 (m, 1H), 2.39–2.44 (m, 2H), 2.49–2.53 (m, 2H), 2.61–2.68 (m, 1H), 3.72–3.85 (m, 1H), 4.05–4.11 (m, 2H), 4.85–4.89 (m, 2H), 5.02–5.11 (m, 4H), 7.05–7.29 (m, 25H). ^{13}C NMR (75 MHz, CD_3OD): δ 29.93, 31.20, 31.26, 34.06, 47.03, 47.13, 55.17, 67.49, 67.70, 67.91, 67.95, 69.53, 127.23, 128.90, 129.59, 129.72, 137.26, 137.53, 137.63, 137.73, 137.83, 174.10, 174.11, 174.46. ^{31}P NMR (121 MHz, CD_3OD): δ 5.20, 5.26, 5.31, 5.36. Anal. Calcd for $C_{44}H_{46}NO_9P$: C, 69.19; H, 6.07; N, 1.83. Found: C, 69.07; H, 5.91; N, 1.75.

4.16. 2-[(2-Carboxy-4-phenyl-butoxy)-hydroxy-phosphorylamino]-pentanedioic acid tetrapotassium salt (1f) (derived from 7f using general procedure B)

^{1}H NMR (300 MHz, D_2O): δ 1.76–1.96 (m, 4H), 2.19–2.24 (m, 2H), 2.62–2.69 (m, 3H), 3.70–3.83 (m, 2H), 4.07–4.12 (m, 1H), 7.25–7.39 (m, 5H). ^{13}C NMR (75 MHz, D_2O): δ 28.86, 30.36, 30.56, 32.96, 33.43, 34.38, 47.84, 47.95, 53.43, 55.50, 55.90, 65.25, 126.32, 128.85, 142.23, 142.54, 176.50, 178.99, 182.49. ^{31}P NMR (121 MHz, D_2O): δ 1.18 and 1.26. FAB-HRMS $(M-K)^-$ Calcd 515.9630. Found: 515.9627 for $C_{16}H_{18}K_3NO_9P$.

4.17. 2-[Benzyloxy-(2-benzyloxycarbonyl-ethoxy)-phosphorylamino]-pentanedioic acid dibenzyl ester (7g) (using general procedure A)

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.82–2.18 (m, 2H), 2.37–2.42 (m, 2H), 2.61–2.69 (m, 2H), 3.35–3.39 (m, 1H), 3.87–4.01 (m, 1H), 4.20–4.27 (m, 2H), 4.92–4.96 (m, 2H), 5.05–5.12 (m, 6H), 7.26–7.31 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.98, 30.05, 30.54, 36.04, 36.14, 54.44, 62.75, 67.09, 67.20, 68.01, 68.90, 128.44, 128.51, 128.93, 129.01, 129.24, 129.30, 135.89, 136.37, 136.53, 168.98, 170.87, 173.05. ^{31}P NMR (121 MHz, $CDCl_3$): δ 4.46 and 4.50. FAB-HRMS $(M+H)^+$ Calcd 660.2362. Found: 660.2353 for $C_{36}H_{39}NO_9P$.

4.18. 2-[(2-Carboxy-ethoxy)-hydroxy-phosphorylamino]-pentanedioic acid tetrapotassium salt (1g) (derived from 7g using general procedure B)

^{1}H NMR (300 MHz, D_2O): δ 1.85–1.95 (m, 1H), 2.00–2.08 (m, 1H), 2.21–2.31 (m, 2H), 2.59–2.63 (m, 2H), 3.92–4.01 (m, 2H), 4.10–4.14 (m, 1H). ^{13}C NMR (75 MHz, D_2O): δ 29.48, 35.07, 38.50, 56.29, 61.23, 174.66, 179.87, 183.13. ^{31}P NMR (121 MHz, D_2O): δ 1.29. FAB-HRMS $(M-K)^-$ Calcd 411.9004. Found: 411.9020 for $C_8H_{10}K_3NO_9P$.

4.19. 2-[Benzyloxy-(4-phenyl-butoxy)-phosphorylamino]-pentanedioic acid dibenzyl ester (7h) (using general procedure A)

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61–1.65 (m, 4H), 1.90–1.95 (m, 1H), 2.05–2.11 (m, 1H), 2.38–2.44 (m, 2H), 2.58–2.60 (m, 2H), 3.34 (t, 1H, J = 9.0 Hz), 3.92–3.99 (m, 2H), 4.94–5.10 (m, 6H), 7.14–7.34 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.88, 29.97, 30.04, 30.48, 35.95, 54.37, 67.06, 67.23, 67.29, 67.94, 68.64, 68.72, 68.76, 68.83, 126.47, 128.42, 128.85, 128.88, 128.97, 129.01, 129.18, 129.28, 135.81, 136.44, 137.01, 142.61, 173.01, 173.21, 173.27. ^{31}P NMR (121 MHz, $CDCl_3$): δ 4.65. Anal. Calcd for $C_{36}H_{40}NO_7P$: C, 68.67; H, 6.40; N, 2.22. Found: C, 68.60; H, 6.36; N, 2.12.

4.20. 2-[Hydroxy-(4-phenyl-butoxy)-phosphorylamino]-pentanedioic acid (1h) (derived from 7h using general procedure B)

^{1}H NMR (300 MHz, D_2O): δ 1.60–1.67 (m, 4H), 1.71–1.79 (m, 2H), 2.19–2.22 (m, 2H), 2.65 (t, 1H, J = 6.9 Hz), 3.45–3.52 (m, 1H), 3.76–3.78 (m, 2H), 7.25–7.36 (m, 5H). ^{13}C NMR (75 MHz, D_2O): δ 27.30, 29.73, 32.25, 33.99, 34.89, 56.86, 65.09, 126.09, 128.82, 143.42, 181.66, 183.24. ^{31}P NMR (121 MHz, D_2O): δ 4.74 and 4.81. FAB-HRMS $(M-K)^-$ Calcd 434.0173. Found: 434.0202 for $C_{15}H_{19}K_3NO_7P$.

4.21. 2-Benzoylamino-3-(bis-benzyloxy-phosphoryloxy)-propionic acid benzyl ester (7i)

A mixture of benzoylamino-3-hydroxy-propionic acid benzyl ester (**4a**) (0.815 g, 2.72 mmol) and 5-ethylthio-1*H*-tetrazole (0.886 g, 6.8 mmol) was dissolved in

CH_3CN under argon. Then dibenzyl diisopropyl phosphoramidite (2550 μL, 7.75 mmol) was added dropwise via syringe, and the resulting solution was stirred for 9 h. The solution was then oxidized by adding *tert*-butyl hydroperoxide (3 mL, 21.67 mmol) and stirred for 45 m. The crude product was taken up in CH_2Cl_2, washed twice with 10% HCl (50 mL), 10% $NaHCO_3$ (50 mL), H_2O (50 mL), brine (50 mL), and then dried over $MgSO_4$. The product was isolated by silica gel flash chromatography (1:3 acetone/hexane) as a viscous oil (21%). ^1H NMR (300 MHz, $CDCl_3$): δ 4.32–4.55 (m, 2H), 4.92–5.02 (m, 5H), 5.20 (s, 2H), 7.26–7.67 (m, 18H), 7.85 (d, 2H, $J = 13.5$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 54.20, 54.26, 68.08, 68.40, 70.47, 128.00, 128.59, 128.68, 128.91, 129.17, 129.27, 129.42, 132.54, 134.05, 136.07, 167.79, 169.50. ^{31}P NMR (121 MHz, $CDCl_3$): δ 0.45. FAB-HRMS $(M+H)^+$ Calcd 560.1838. Found: 560.1812 for $C_{31}H_{31}NO_7P$.

4.22. 2-Benzoylamino-3-phosphonooxy-propionic acid tripotassium salt (1i) (derived from compound 7i using general procedure B)

Yield 89%. ^1H NMR (300 MHz, D_2O): δ 4.25–4.31 (m, 2H), 4.62 (s, 1H), 7.51–7.62 (m, 3H), 7.85 (d, 2H, $J = 6$ Hz). ^{13}C NMR (75 MHz, D_2O): δ 56.41, 65.70, 127.57, 128.96, 132.45, 133.55, 170.59, 175.71. ^{31}P NMR (121 MHz, D_2O): δ 0.87. FAB-HRMS $(M-K)^-$ Calcd 363.9391. Found: 363.9409 for $C_{10}H_9K_2NO_7P$.

4.23. PSMA inhibition assay

Inhibition studies were performed as described previously with only minor modifications.[24] Working solutions of the substrate (*N*-[4-(phenylazo)benzoyl]-glutamyl-γ-glutamic acid, PAB-Glu-γ-Glu) and all inhibitors were prepared in Tris buffer (50 mM, pH 7.4). Working solutions of purified PSMA were appropriately diluted in Tris buffer (50 mM, pH 7.4) to provide from 15% to 20% conversion of substrate to product in the absence of inhibitor. A typical incubation mixture (final volume 250 μL) was prepared by the addition of either 25 μL of an inhibitor solution or 25 μL Tris buffer (50 mM, pH 7.4) to 175 μL Tris buffer (50 mM, pH 7.4) in a test tube. A volume of the 25 μL PAB-Glu-γ-Glu (100μM) was added to the above solution. The enzymatic reaction was initiated by the addition of 25 μL of the PSMA working solution. In all cases, the final concentration of PAB-Glu-γ-Glu was 10 μM while the enzyme was incubated with five serially diluted inhibitor concentrations to provide a range of inhibition from 10% to 90% inhibition. The reaction was allowed to proceed for 15 min with constant shaking at 37 °C and was terminated by the addition of 25 μL methanolic TFA (2% trifluoroacetic acid by volume in methanol) followed by vortexing. The quenched incubation mixture was quickly buffered by the addition of 25 μL K_2HPO_4 (0.1 M), vortexed, and centrifuged (10 min at 7000*g*). An 85 μL aliquot of the resulting supernatant was subsequently quantified by HPLC as previously described.[18] IC_{50} values were calculated using KaleidaGraph 3.6 (Synergy Software).

4.24. Computational modeling

Prior to docking, ligands were minimized in the MMFF94 force field using SZYBKI (OpenEyes) and then converted into conformational libraries using OMEGA (OpenEyes).[26] Docking was then performed with FRED2 (OpenEyes) employing a structure of recombinant PSMA extracellular domain co-crystallized with a phosphonate inhibitor (PDB = 2C6C).[16] Partial charges were assigned with MOLCHARGE (OpenEyes) using the MMFF94 model for the protein and the AM1BCC model for ligands. The active site was defined as the residues falling within a 12 Å sphere around the PSMA catalytic zinc atoms. Inhibitor conformations non-productive for PSMA inhibitory activity were filtered by utilizing a restraint in the docking model. Namely, the phosphoramidate functional group, known to be a zinc-binding group (ZBG), was constrained through an appropriate SMARTS string to be within 2 Å of the center of the two PSMA zinc atoms. For each substrate, the top scoring consensus pose was identified using two FRED2 scoring functions which consider metal interactions between the protein and ligand (ChemGauss2 and PLP). Employing this pose, the protein–ligand complex was subsequently re-minimized in the MMFF94 force field as implemented in SZYBKI, without restraint. The final protein–ligand complexes were visualized using PYMOL (DeLano Scientific).

Acknowledgments

This work was supported in part by grants from the National Institutes of Health, MBRS SCORE Program-NIGMS (Grant No. S06-GM052588) and the Department of Defense (Grant No. PC051060). The authors would also like to extend their gratitude to W. Tam and the NMR facility at SFSU for their expert assistance.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.07.028.

References and notes

1. *Drugs in R&D* **2004**, *5*.
2. Carter, R. E.; Feldman, A. R.; Coyle, J. T. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 749–753.
3. Tiffany, C. W.; Lapidus, R. G.; Merion, A.; Calvin, D. C.; Slusher, B. S. *Prostate* **1999**, *39*, 28–35.
4. Holmes, E. H.; Greene, T. G.; Tino, W. T.; Boynton, A. L.; Aldape, H. C.; Misrock, S. L.; Murphy, G. P. *Prostate Suppl.* **1996**, *7*, 25–29.
5. Horoszewicz, J. S.; Leong, S. S.; Kawinski, E.; Karr, J. P.; Rosenthal, H.; Chu, T. M.; Mirand, E. A.; Murphy, G. P. *Cancer Res.* **1983**, *43*, 1809–1818.
6. Bacich, D. J.; Pinto, J. T.; Tong, W. P.; Heston, W. D. *Mamm. Genome* **2001**, *12*, 117–123.

7. Chang, S. S.; O'Keefe, D. S.; Bacich, D. J.; Reuter, V. E.; Heston, W. D.; Gaudin, P. B. *Clin. Cancer Res.: An Official Journal of the American Association for Cancer Research* **1999**, *5*, 2674–2681.
8. Chang, S. S.; Reuter, V. E.; Heston, W. D.; Gaudin, P. B. *Urology* **2001**, *57*, 801–805.
9. Tasch, J.; Gong, M.; Sadelain, M.; Heston, W. D. *Crit. Rev. Immunol.* **2001**, *21*, 249–261.
10. Salit, R. B.; Kast, W. M.; Velders, M. P. *Frontiers Biosci.: A Journal and Virtual Library* **2002**, *7*, e204–e213.
11. Lu, J.; Celis, E. *Cancer Res.* **2002**, *62*, 5807–5812.
12. Fracasso, G.; Bellisola, G.; Cingarlini, S.; Castelletti, D.; Prayer-Galetti, T.; Pagano, F.; Tridente, G.; Colombatti, M. *The Prostate* **2002**, *53*, 9–23.
13. Heston, W. D. *Urology* **1997**, *49*, 104–112.
14. Pinto, J. T.; Suffoletto, B. P.; Berzin, T. M.; Qiao, C. H.; Lin, S.; Tong, W. P.; May, F.; Mukherjee, B.; Heston, W. D. *Clin. Cancer Res.* **1996**, *2*, 1445–1451.
15. Conway, R. E.; Petrovic, N.; Li, Z.; Heston, W.; Wu, D.; Shapiro, L. H. *Mol. Cell. Biol.* **2006**, *26*, 5310–5324.
16. Mesters, J. R.; Barinka, C.; Li, W.; Tsukamoto, T.; Majer, P.; Slusher, B. S.; Konvalinka, J.; Hilgenfeld, R. *EMBO J.* **2006**, *25*, 1375–1384.
17. Davis, M. I.; Bennett, M. J.; Thomas, L. M.; Bjorkman, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 5981–5986.
18. Maung, J.; Mallari, J. P.; Girtsman, T. A.; Wu, L. Y.; Rowley, J. A.; Santiago, N. M.; Brunelle, A. N.; Berkman, C. E. *Bioorg. Med. Chem.* **2004**, *12*, 4969–4979.
19. Jackson, P. F.; Cole, D. C.; Slusher, B. S.; Stetz, S. L.; Ross, L. E.; Donzanti, B. A.; Trainor, D. A. *J. Med. Chem.* **1996**, *39*, 619–622.
20. Lu, H. Y.; Ng, R. J.; Shieh, C. C.; Martinez, A. R.; Berkman, C. E. *Phosphorus Sulfur Silicon Relat. Elem.* **2003**, *178*, 17–32.
21. Sunazuka, T.; Tsuzuki, K.; Kumagai, H.; Tomoda, H.; Tanaka, H.; Nagashima, H.; Hashizume, H.; Omura, S. *J. Antibiot.* **1992**, *45*, 1139–1147.
22. Pfeffer, P. E.; Silbert, L. S.; Kinsel, E. *J. Org. Chem.* **1972**, *37*, 1256.
23. Wu, L. Y.; Berkman, C. E. *Tetrahedron Lett.* **2005**, *46*, 5301–5303.
24. Wone, D. W.; Rowley, J. A.; Garofalo, A. W.; Berkman, C. E. *Bioorg. Med. Chem.* **2006**, *14*, 67–76.
25. Liu, T.; Toriyabe, Y.; Berkman, C. E. *Protein Expr. Purif.* **2006**, *49*, 251–255.
26. Bostrom, J.; Greenwood, J. R.; Gottfries, J. *J. Mol. Graph. Model.* **2003**, *21*, 449–462.